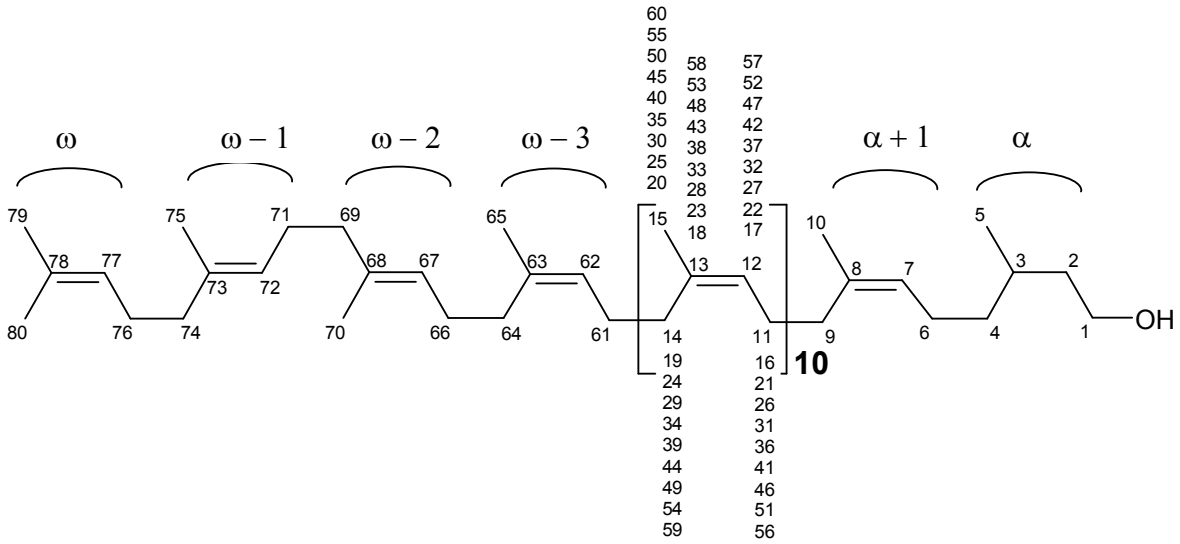
Supplemental Data

Supplemental Figure 1. Structure of Dol-16. Sequential numbering of carbon atoms is indicated (see also Supplemental Table 1), α and ω stand for the terminal isoprene units.



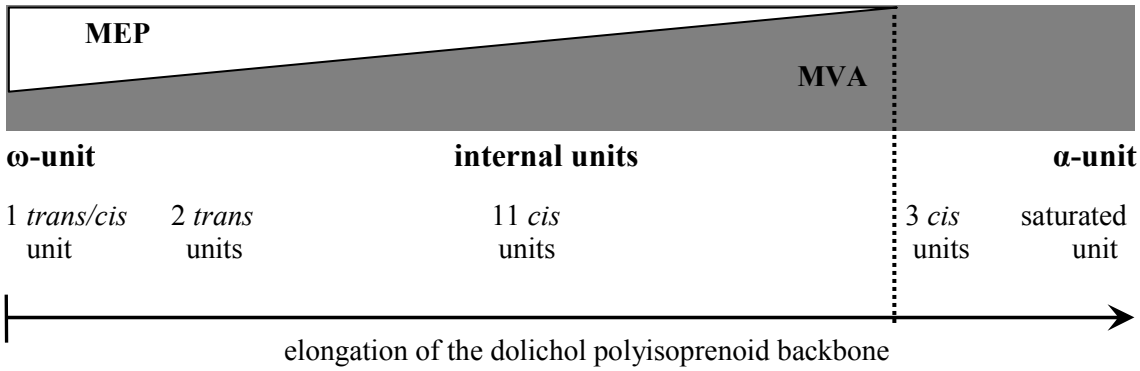
Supplemental Figure 2. Involvement of the MEP and MVA pathways in biosynthesis of dolichols. Dol-18 is shown as an example. Dotted line separates the α-terminal segment of the dolichol molecule derived exclusively from the MVA pathway from the mixed-pathway core. For each Dol-n the mixed-pathway core cannot be longer than 13 isoprene units, since the α-terminal isoprene unit of Dol-14, the shortest dolichol, is of the MVA-origin.

The data on the biosynthetic origin of the respective isoprene units were obtained from the following experiments:

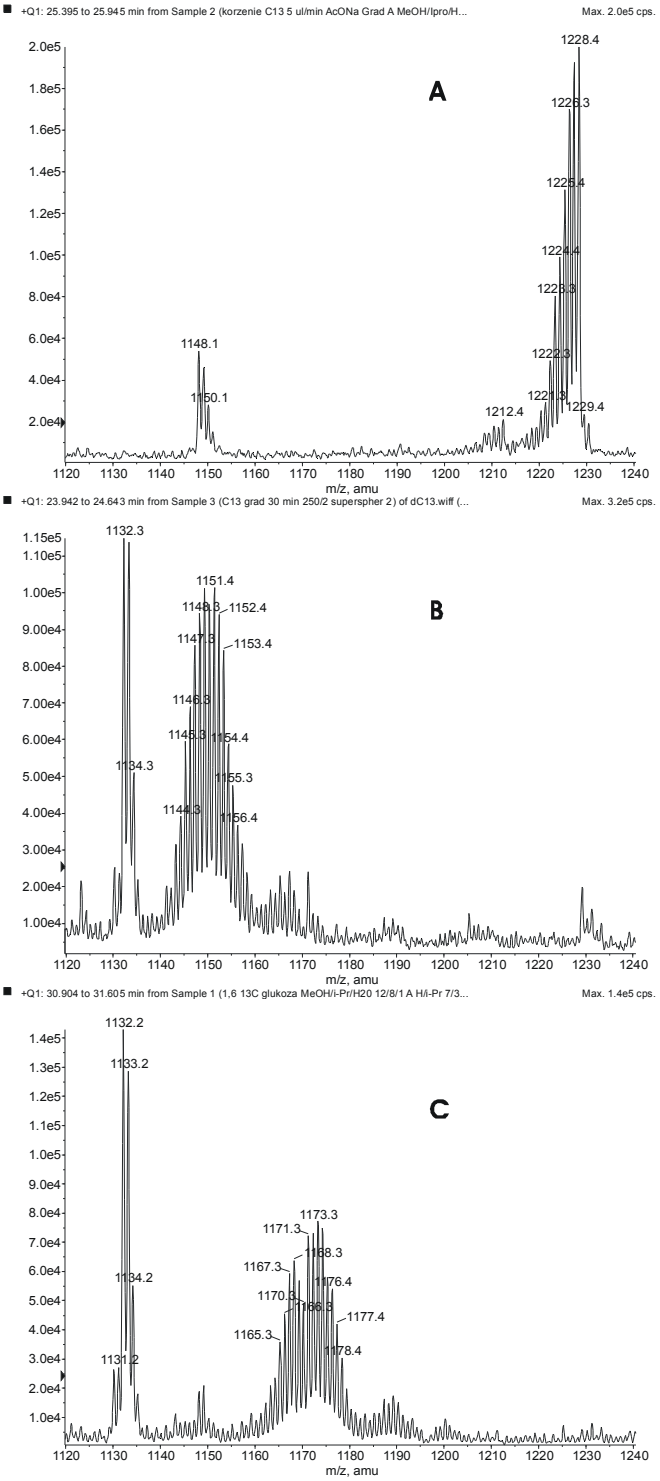
ω-terminal, *trans* and internal *cis* isoprene units - ^{13}C-NMR,

α-terminal units - ^{13}C-NMR and MS,

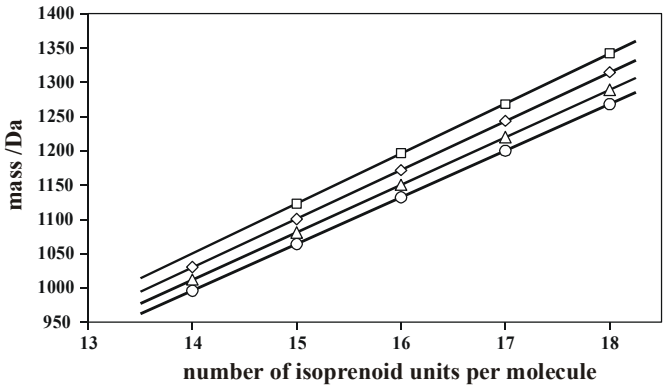
approximate ratio of the involvement of the MEP and MVA pathways - MS.



Supplemental Figure 3. Labeling of dolichol by [^{13}C]-labeled glucose. Shown are HPLC/ESI-MS spectra of Dol-16 obtained from: A, [U-$^{13}C_6$]glucose – in this case adducts with potassium were recorded; B, [1-^{13}C]glucose; C, [1,6-$^{13}C_2$]glucose. The group of peaks to the left in each panel corresponds to unlabeled dolichol coming from root inoculum.



Supplemental Figure 4. The effect of ^{13}C –labeling on the molecular mass of dolichols of different lengths. Estimated average masses of dolichols (Supplemental Table 2) were plotted as a function of the number of their isoprene units. Each data point represents average molecular mass of a particular dolichol obtained from feeding with a specifically labeled glucose: circles, native glucose; triangles, [1-^{13}C]glucose; diamonds, [1,6-$^{13}C_2$]glucose; squares, [U-$^{13}C_6$]glucose. Solid lines represent results of regression analysis (c.f. Table 3 for details).



Regression lines obtained in Supplemental Fig. 4 are described by the general formula /2/ (see Methods for detailed description):

$$M(n,i) = Dol_o + n * m(i) \qquad /2/$$

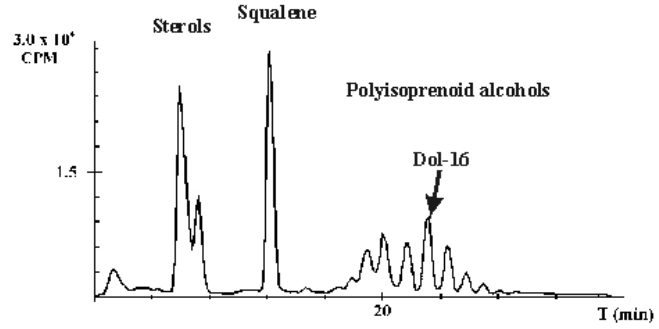
Equations describing the regression lines for the four experimental conditions are as follows:

(i=1) $M_n = 68.08(0.05)n + 43.1(0.2)$ native glucose
(i=2) $M_n = 69.32(0.09)n + 41.4(1.5)$ [1-^{13}C]glucose
(i=3) $M_n = 71.17(0.21)n + 33.7(3.4)$ [1,6-$^{13}C_2$]glucose
(i=4) $M_n = 72.87(0.42)n + 45.5(6.9)$ [U-$^{13}C_6$]glucose

Parameters of these equations - four slope values (Standard Error included) and four intercept values (SE included) are summarized in main text in Table 3.

As described in Results the estimated enrichment of the mass of the isoprene units (Table 3) clearly indicated their origin from the MVA pathway. Interestingly, the intercept values, Dol_0, were lower than expected (Table 3). One, universal value of the mass of Dol_0 in all the experiments should be expected (see the chemical formula below Table 3 and Experimental procedures for details). Therefore estimated deficit of the molecular mass of Dol_0 can only be explained by the fact that the average molecular mass of dolichol pseudomolecular ion is lower than expected for the MVA-origin. This is possible only if some isoprenoid units constituting dolichol molecules are 'lighter' (derived from the MEP pathway, up to two ^{13}C atoms per unit) than the 'heavy' α –terminal isoprene units derived from the MVA pathway (up to three ^{13}C atoms per unit).

Supplemental Figure 5. Incorporation of [^{3}H]mevalonate into lipids extracted from *Coluria* roots. HPLC/radiometric spectrum signals are identified by comparison with unlabeled standards.



Supplemental Table 1. Correlation of carbon atom numbers in Dol-16 molecule and their positions in IPP

position in IPP	carbon atom type	chemical shift (ppm)	carbon atom number in dolichol chain
	CH_2 ω	27.2	76
	CH_2 *trans* ω-1, ω-2	27.1	66,71
C-1	CH_2 *cis*	26.9	6,11,16,21,26,31, 36,41,46,51,56,61
	CH_2 α	60.8	1
	CH ω	124.9	77
	CH *trans* ω-1, ω-2	124.7 124.8	67,72
C-2	CH *cis*	125.6	12,17,22,27,32,37, 42,47,52,57,62
	CH *cis* α +1	126.2	7
	CH_2 α	40.3	2
	C ω	131.1	78
	C *trans* ω-1	135.4	73
	C *cis*	135.3	13,18,23,28,33,38,
C-3			43,48,53,58,63
	C *trans* ω-2	134.8	68
	C *cis* α+1	135.0	8
	CH α	29.5	3
	CH_3 ω *trans*	17.7	79
	CH_2 *trans* ω-1, ω-2	40.2	69,74
	CH_2 *cis* ω-3	32.4	64
C-4	CH_2 *cis*	32.6	9,14,19,24,29,34, 39,44,49,54,59
	CH_2 α	37.9	4
	CH_3 ω *cis*	25.8	80
	CH_3 *trans* ω-1, ω-2	16.1	70,75
C-5	CH_3 *cis*	23.7	10,15,20,25,30,35, 40,45,50,55,60,65
	CH_3 α	19.7	5

Supplemental Table 2. Expected and experimental average molecular masses of dolichols. Experimental values obtained from fitting of Gaussian distribution to the experimentally recorded profile of isotopomer distribution (see Fig. 5) and expected values estimated separately for the MEP and the MVA pathway according to their theoretical enrichments (see Table 3).

		Average molecular mass		
Feeding experiment	Dolichol	Experimental	MEP expected	MVA expected
native glucose	Dol-14	996.1	996.1	
	Dol-15	1064.1	1064.2	
	Dol-16	1132.2	1132.3	
	Dol-17	1200.3	1200.4	
	Dol-18	1268.4	1268.4	
[1-^{13}C]glucose	Dol-14	1012.1	1009.8	1016.8
	Dol-15	1080.9	1078.9	1086.4
	Dol-16	1150.4	1148.0	1156.0
	Dol-17	1220.2	1217.0	1225.5
	Dol-18	1289.0	1286.1	1295.1
[1,6-$^{13}C_2$]glucose	Dol-14	1030.7	1023.3	1036.9
	Dol-15	1100.4	1093.3	1107.8
	Dol-16	1172.0	1163.3	1178.8
	Dol-17	1243.9	1233.3	1249.8
	Dol-18	1314.8	1303.4	1320.8
[U-$^{13}C_6$]glucose	Dol-14		1064.7	
	Dol-15	1138.4	1137.7	
	Dol-16	1212.4	1210.7	
	Dol-17	1283.5	1283.7	
	Dol-18	1357.6	1356.6	

Supplemental Text – Part 1 *Unambiguous estimation of the number of isoprene units in the Dol-n molecule derived from either pathway*

The value of the average molecular mass (M) recorded for Dol-n during labeling experiment permits unambiguous estimation of the number of isoprene units derived from either pathways.

This problem is mathematically equivalent to a system of two equations with two unknown variables, k_{MEP} and k_{MVA}, to be resolved:

$$\begin{cases} k_{MEP} + k_{MVA} = n \\ M_n = M_{nat} + k_{MEP} * \varepsilon_{MEP} + k_{MVA} * \varepsilon_{MVA} \end{cases}$$

k_{MEP}, number of isoprene units synthesized via the MEP pathway,
k_{MVA}, number of isoprene units synthesized via the MVA pathway,
n, total number of isoprene units in Dol-n molecule,
ε_{MEP}, the enrichment of the mass of isoprene unit expected for the MEP pathway at given labeling conditions (see main text Table 3),
ε_{MVA}, the enrichment of the mass of isoprene unit expected for the MVA pathway at given labeling conditions (see main text Table 3),
M_{nat}, molecular mass of native Dol-n estimated by MS measurement,
M_n, molecular mass estimated by MS measurement at given labeling conditions.

This approach was used for estimation of the ($k = k_{MEP}$) and ($n-k = k_{MVA}$) in Results and Supplemental Text – part 2.

Such reasoning is possible with following restrictions:

- the value of *m/z* obtained from the MS measurement is unambiguously ascribed to a well-defined dolichol (i.e., where molecules consist of a given number of isoprene units); in our approach it was ensured by the HPLC/MS method since the mixture of homologous dolichols was separated on the HPLC column and the *m/z* for each homologue was sequentially estimated (reference 3);
- the value of *m/z* corresponds to the molecular mass of dolichol, i.e. the dolichol does not undergo any fragmentation upon (especially) MS analysis (reference 3);
- all the isoprene units within the dolichol molecule are labeled from the biosynthetic precursor [^{13}C]glucose and this is achieved only when the cellular pool of native biosynthetic precursors is negligible – this was shown in the manuscript by [U-$^{13}C_6$]glucose labeling (see Results) - thus only two types of isoprene units are expected, either the MVA- or the MEP-derived ones.

Supplemental Text - Part 2

In order quantitatively estimate the number of the MEP- and MVA-derived isoprene units per Dol-n molecule, general formula /3/ describing the molecular mass of dolichol as a function of the isotopic enrichment was constructed (see Experimental for details):

$$M(\varepsilon, n) = M_{nat}(n) + (n-k) * \varepsilon_{MVA} + k * \varepsilon_{MEP}, \qquad /3/$$

Equations describing the regression lines for the five dolichols shown in main text Fig. 6 are as follows:

(n=14) $M(\varepsilon) = 995.6(1.2) + 7.3(1.8) * \varepsilon_{MVA} + 6.7\,(1.8) * \varepsilon_{MEP}$ Dol-14

(n=15) $M(\varepsilon) = 1063.6(1.2) + 7.1(1.8) * \varepsilon_{MVA} + 8.0(1.8) * \varepsilon_{MEP}$ Dol-15

(n=16) $M(\varepsilon) = 1131.5(1.4) + 8.6(2.4) * \varepsilon_{MVA} + 7.4(2.4) * \varepsilon_{MEP}$ Dol-16

(n=17) $M(\varepsilon) = 1199.5(1.6) + 10.5(2.7) * \varepsilon_{MVA} + 6.5\,(2.7) * \varepsilon_{MEP}$ Dol-17

(n=18) $M(\varepsilon) = 1267.4(2.1) + 11.4(3.3) * \varepsilon_{MVA} + 6.6(3.3) * \varepsilon_{MEP}$ Dol-18

Finally, the values of parameter k calculated from the above equations are summarized in main text Table 4.